STARCHY AND STARCHLESS POLLEN IN THE ONAGRACEAE

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ABSTRACT

Mature pollen from 76 species in the complete roster of genera in the Onagraceae was examined for starch-containing or starchless contents. *Fuchsia* and *Lopezia* pollen grains, after passing through a stage of starch accumulation, lose the starch (presumably converted to lipid) and are classified as "starchless." All other genera have pollen that is starch-containing at maturity. The phylogenetic implications of this are considered. Pollen grain diameter is larger in the "starchy" taxa than in the "starchless" ones. There is a strong correlation of pollen grain diameter and the length of style to be traversed by the pollen tube in species with "starchy" grains.

The presence of abundant starch grains in pollen grains of species of *Oenothera* has been appreciated since the 19th century, and Renner (1919a, 1919b, 1921) showed that the size and shape of the starch grains may give a visible expression of the gene-complexes in those species of *Oenothera* that are "complex heterozygotes." We have reported (Baker & Baker, 1979) that there are families of angiosperms that are characterized by starchy pollen grains (e.g. Gramineae, Geraniaceae) and families in which starch does not occur at the time of anther-dehiscence (e.g. Compositae, Cruciferae), but also there are families in which both "starchy" and "starchless" taxa are found. The Onagraceae is one such family.

The purpose of the present paper is to report upon a survey of the pollen reserves of species in all the genera of the Onagraceae and to consider its adaptive and phylogenetic implications. Of great assistance in this is the availability of a comprehensive survey of the reproductive biology of the family by Raven (1979). The family consists of 17 genera in 7 tribes, with about 674 species (Raven, 1979), and we have been able to study the pollen of some species in each of these genera.

MATERIAL AND METHODS

Live floral material was collected in nature in California, England, and Wales, as well as from cultivated plants in the University of California Botanical Garden, the East Bay Regional Parks Botanic Garden (Contra Costa County, California), and the Strybing Arboretum (San Francisco). Particularly valuable was the supply of fresh material of hard-to-get species by Dr. Peter H. Raven, from the Missouri Botanical Garden, and by Dr. James Estes, of the University of Oklahoma. Herbarium specimens of some of the wild collections are being kept in Berkeley and the various institutions have their own vouchers for material.

Care was taken to sample pollen only from anthers that were freshly dehisced, a procedure which is assisted in the Onagraceae by the tendency of the pollen grains to stick together because of the viscin threads that entangle them (Skvarla et al., 1978). Pollen grains were mounted in iodine dissolved in polyvinyl lactophenol to test for starch on a microscope slide and examined for a period of up to

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ANN. MISSOURI BOT. GARD. 69: 748–754. 1982.

one week. The diameters of at least 10–30 grains were measured with the aid of a micrometer eyepiece (1 division = 2.86 μm). Contents of the pollen grains usually extend into the pores, hence with triporate grains one pore was included in the measurement. When the pollen grains are biporate, as in some species of *Fuchsia*, the mean of the long and short diameters was taken, so that effectively it was the body and one pore. Where the grains are shed in tetrads only the individual grains were measured. "Bad" pollen grains (misshapened or lacking in contents) were noted but not measured. Then, the pollen grains were squashed by gentle pressure on the coverslip, so that the contents exuded into the test solution. This gave a further opportunity of observing starch in small quantity. The presence of globules of oil (originally external or internal) was recorded and the identity of the globules as lipid confirmed where necessary by staining with Sudan IV.

Observations were also made, when possible, of the pollen in various immature stages as well as at maturity.

In a previous survey of many families (Baker & Baker, 1979) it was noted that larger pollen grains tended to be found in flowers with longer styles although no actual measurements of style-length were made at that time to establish a regression. Consequently, for this study, styles were measured on available material. The interest of this was not appreciated initially and it was necessary in some cases to use herbarium material of the species in question that had already been examined as fresh material to acquire measurements of style length. The measurements made were from the middle of the stigma to the top of the ovary. We realize that the pollen tubes that reach to the ovules at the base of the linear ovary have farther to travel than our measurement but, because of the rapid elongation of the ovary during and after pollination, it was not easy to be sure of the longer measurements.

Results

Starchy and Starchless Pollen

Observations and measurements of the pollen grains of 76 species in all of the genera are listed in Tables 1 and 2.

All taxa, with the exception of *Fuchsia* and *Lopezia*, were positive for the presence of starch in pollen grains at anthesis, often in large amounts (Table 1). Although *Fuchsia* and *Lopezia* are designated "starchless" at maturity, a few pollen grains containing a small amount of starch may occur. In tracing the development of these pollen grains it was seen that in all taxa there is a sequence of stages, beginning in very young grains with a starchless condition, followed by an accumulation of starch with, in the cases of *Fuchsia* and *Lopezia* only, a final stage before anthesis of the conversion of starch into lipid. As an example, these stages are associated with anther development of *Fuchsia magellanica* in Table 3.

Pollen Diameter and Starchiness

There is a great range of pollen grain diameters in the family, from 37.2 μm in *Fuchsia thymifolia* to 194.5 μm in *Oenothera caespitosa*. However, the gen-

TABLE 1. Species with pollen containing starch.

	Diameter of Pollen Grains (μm)	Style Length (in mm)
Tribe Jussiaceae		
Ludwigia		
curtisiae	42.9	1.5
octovalvis	77.2	4.0
peploides	77.9	8.0
uruguayensis	105.8	9.5
virgata	57.2	1.9
Tribe Circaeeae		
Circaea		
cordata	45.8	4.3
lutetiana	42.9	4.0
Tribe Hauyeae		
Hauya heydeana	103.0	49.0
Tribe Onagreae		
Gongylocarpus rubricaulis	108.7	4.8
Gayophytum diffusum		
ssp. *diffusum*	82.9	4.0
spp. *parviflorum*	71.5	2.6
Xylonagra arborea	94.4	30.0
ssp. *wigginsii*	95.1	30.7
Camissonia		
angelorum	80.1	11.2
boothii ssp. *decorticans*	94.4	9.1
cardiophylla	123.0	16.5
cheiranthifolia	111.5	9.7
ovata	117.3	10.8
Calylophus toumeyi	128.7	53.1
Gaura		
heterandra	128.7	4.0
parviflora	111.5	3.0
Oenothera		
caespitosa	194.5	154.3
deltoides	124.4	96.0
elata	165.9	64.0
macrosceles	151.6	
organensis	191.6	151.5
pilosella ssp. *sessilis*	157.6	
rosea	94.4	14.0
stubbei	160.2	
Stenosiphon linifolius	94.4	8.5
Clarkia		
breweri	157.3	41.5
concinna	160.2	31.0
deflexa	165.9	19.7
gracilis var. *gracilis*	123.0	10.3
purpurea	120.2	11.1
rubicunda	128.7	19.3
unguiculata	117.3	8.0
williamsonii	140.1	20.8
Heterogaura heterandra	65.8	5.6

TABLE 1. Continued.

	Diameter of Pollen Grains (μm)	Style Length (in mm)
Tribe Epilobieae		
Boisduvalia		
cleistogama	48.6	2.3
densiflora	54.3	4.3
macrantha	91.5	5.3
Epilobium		
alpinum	54.3	4.5
angustifolium	77.2	16.2
brunescens ssp. *brunescens*	58.6	4.1
canum	111.5	37.4
ssp. *angustifolium*	131.6	39.0
ssp. *garrettii*	120.1	
ssp. *latifolium*	123.0	38.4
ssp. *septentrionalis*	108.7	36.8
ciliatum ssp. *ciliatum*	82.9	4.7
glabellum	91.5	
hirsutum	100.1	12.3
hormannii	60.1	4.7
lanceolatum	74.4	6.3
melanocephalum	68.4	
minutum	70.1	2.1
montanum	85.8	7.5
paniculatum	82.9	3.1
?*saximontanum*	65.8	
Mean	102.9	

eralization made for flowering plants by Baker and Baker (1979) holds for the Onagraceae: the mean diameter of pollen grains of taxa that show starchy pollen ($\bar{x}$ = 102.9 μm; range = 42.9 to 194.5 μm; n = 60) is larger than the mean of those with starchless pollen ($\bar{x}$ = 69.0 μm; range = 37.2 to 144.4 μm; n = 16). Standard deviations are not quoted because the data do not have a "normal" distribution. Application of the Wilcoxon two-sample test (adjusted for tied values) gives t_s = 3.174 (d.f. 73) which is significant at the .005 level.

POLLEN DIAMETER AND LENGTH OF STYLE

Tables 1 and 2 also contain the mean style lengths for those species available for the measurement. Analysis of these data for starchy pollen grains (for pollen grain diameter compared with the $\log_{10}$ of style length) by ANOVA showed that the positive correlation is highly significant ($F_{(1,51)} = 81.653$, where $F_{.001(1,51)} = 12.51$). Figure 1 shows this relationship for all the species analyzed and the regression line that fits these data. With a correlation coefficient r = 0.785, almost 62% of the variation observed can be explained by this relationship.

A similar analysis for starchless pollen would probably show a similar relationship but insufficient style-length data have been available for statistical investigation.

TABLE 2. Species with pollen not containing starch.

	Diameter of Pollen Grains (μm)	Style Length (in mm)
Tribe Fuchsieae		
Fuchsia		
boliviana	62.9	57.1
collensoi	63.6	
excorticata	62.9	
magellanica	57.2	34.9
microphylla	51.5	
paniculata	45.8	
perscandens	60.1	
pilosa	60.1	
procumbens	57.2	
regia	71.5	
splendens	65.8	
thymifolia	37.2	27.0
Tribe Lopezieae		
Lopezia		
ciliatula	68.6	71.5
langmanniae	124.4	131.6
longiflora	144.4	143.0
racemosa	71.5	
Mean	69.0	

DISCUSSION

As in our general survey of starchy and starchless pollen in a number of families (Baker & Baker, 1979), we find in the Onagraceae that the mean pollen grain diameter of species that produce starchy pollen is significantly greater than that of the species that produce starchless (oil-rich) pollen.

In the same preliminary survey of pollen grains of many families, their contents and their sizes (Baker & Baker, 1979), we drew attention to the significantly larger pollen grains of species with styles longer than 1 cm, compared to those produced by species with styles shorter than this length. However, recently,

TABLE 3. Changes in percentage of pollen grains containing starch in *Fuchsia magellanica* during development.

Stage	Pollen Grain Diameter (μm)	Percentage	
		+ (for starch)	− (for starch)
1. Very young anthers white	52.9	0.7	99.3
2. Anthers with slight tinge of red	54.3	16	84
3. Anthers pale pink	55.8	82.6	17.4
4. Anther wall deep pink; flower not open	55.8	68	32
5. Anthers just beginning to dehisce; flower just beginning to open	57.2	15*	85
6. Open flower	57.2	<1*	>99

* Starch grains that are present are very small.

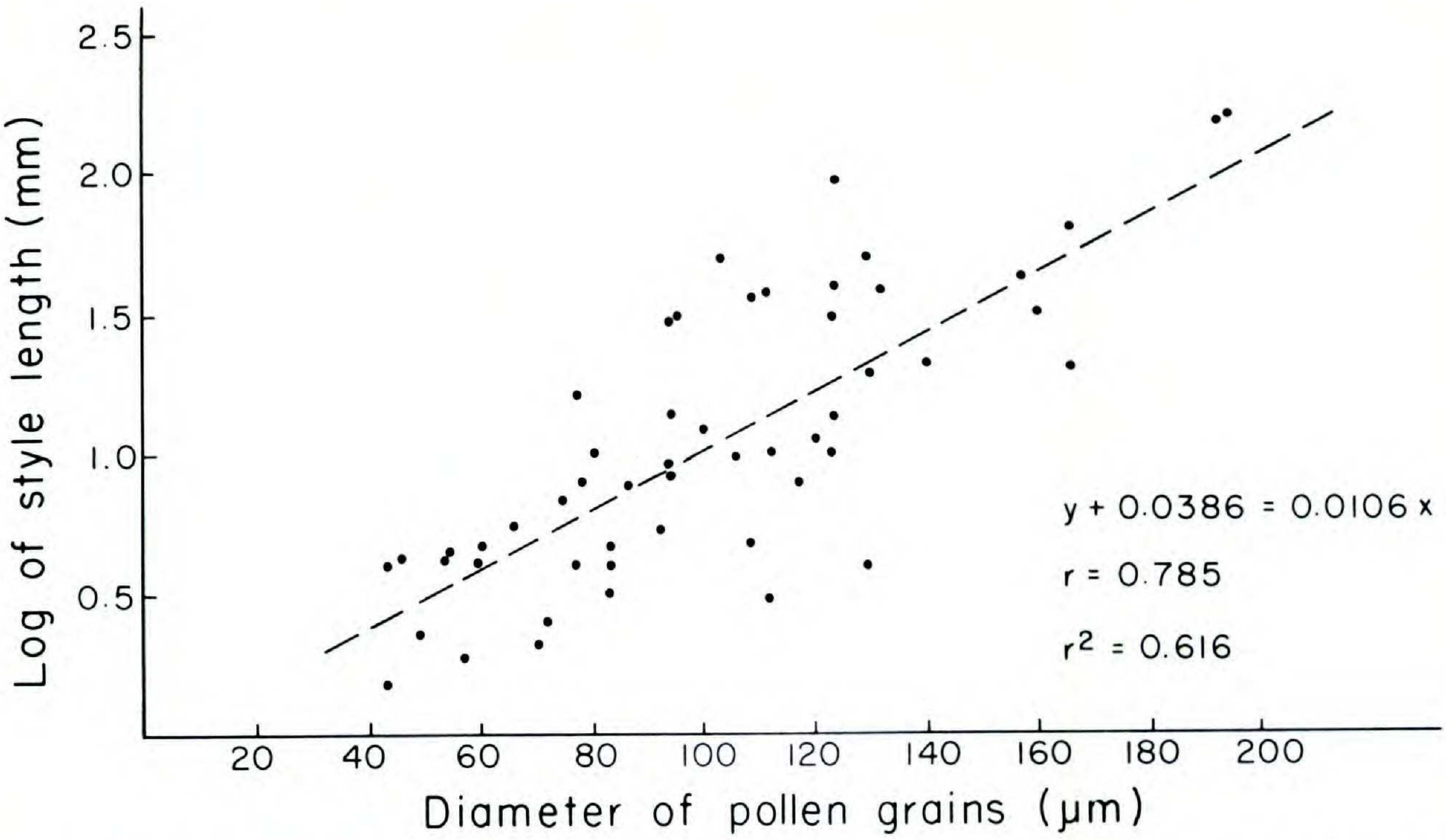

FIGURE 1. Relation of pollen grain diameter and $\log_{10}$ style-length for 53 species with starchy pollen, and calculated regression line.

Cruden and Miller-Ward (1981) have cast doubt on the likelihood that there would be a correlation between pollen grain size and the length of the pollen tube that must be produced to traverse the style from stigma to ovary. They based this belief largely on the supposition that nutrients will be picked up from the style as the tube grows. Undoubtedly, this occurs and, undoubtedly, there is not always a complete correlation between the size of a pollen grain and the amount of "tube-growing" chemicals within it, but, if the species are taxonomically related and if the food reserve is of the same nature, and even if a proportion of the nutrients needed for pollen-tube growth comes from the style, there may be expected to be some correlation of pollen grain diameter with style-length. Our data for 53 onagraceous species (with starchy pollen) show an excellent correlation. The few data relating to starchless grains (*Fuchsia* and *Lopezia*) suggest that a similar correlation, based on smaller diameters overall, would be found.

According to Raven (1979) the genus *Ludwigia* represents a branch at the most primitive level from the Onagraceae stock (see, also, Eyde, 1978, 1979; Raven & Tai, 1979). This offshoot probably occurred in South America. The genus *Fuchsia* also probably had a Southern Hemisphere origin (with South America, again, the probable ancestral home). *Fuchsia* may be the "most generalized" member of the family (i.e. most like the common ancestor of the family, with the exception of *Ludwigia*) (Raven, 1979). *Lopezia* appears to have been developed from the onagraceous stock (i.e. from a common ancestor with *Fuchsia*) when it reached North America. Raven suggests that many subsequent dispersal acts distributed the Onagraceae around the world.

It is difficult to fit the pollen reserves data with these phylogenetic and phytogeographic pictures. Starchy pollen in *Ludwigia* suggests that the ancestral condition in the Onagraceae was a starchy one (starchy and starchless pollen are both represented in other families of the Myrtales—Baker & Baker, 1979). However, the "starchlessness" of mature *Fuchsia* and *Lopezia* pollen, contrasting with the putatively derived genera of the rest of the family, suggests that starchy pollen is itself a derived condition in the Onagraceae. Starchlessness of *Fuchsia* pollen is not of recent evolution because it is found in the studied representatives of this genus from South and North America and from New Zealand.

No correlation of the nature of pollen reserves with pollinator type is available to explain the starchy or starchlessness of Onagraceae. "Starchless" *Fuchsia* has bird-pollinated, bee-pollinated, fly-pollinated, and butterfly-pollinated species, while *Lopezia* has a similar range of "legitimate" flower-visitors. On the other hand, the "starchy" species of the rest of the family have a range of pollinators that is as wide or wider, including several kinds of moths (Raven, 1979: table 8). Anemophily (wind-pollination), which is frequently associated with starchy pollen (Baker & Baker, 1979), does not occur in the Onagraceae. Autogamy, which is frequently associated with starchy pollen, does occur in many genera and has probably evolved repeatedly (Raven, 1979).

The most likely phylogeny seems to us to specify that the ancestor of the family had starchless pollen but that in *Ludwigia* and in the rest of the family (after *Fuchsia* and *Lopezia* had differentiated) there were switches to "starchiness" by elimination of or restriction of the last stage in the pollen ontogeny, the replacement of starch by lipids. But an explanation of the change on the basis of natural selection cannot yet be given.

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